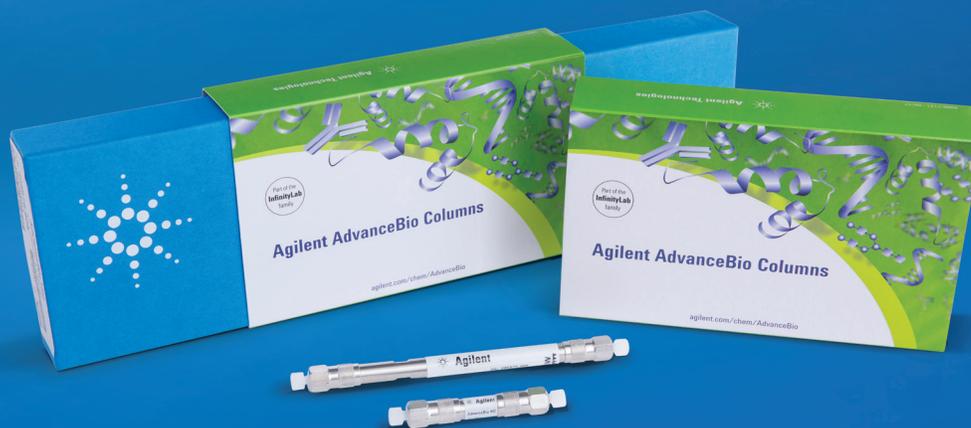


# High-Resolution Analysis of Peptides and Proteins

New AdvanceBio SEC 120 Å 1.9 µm columns

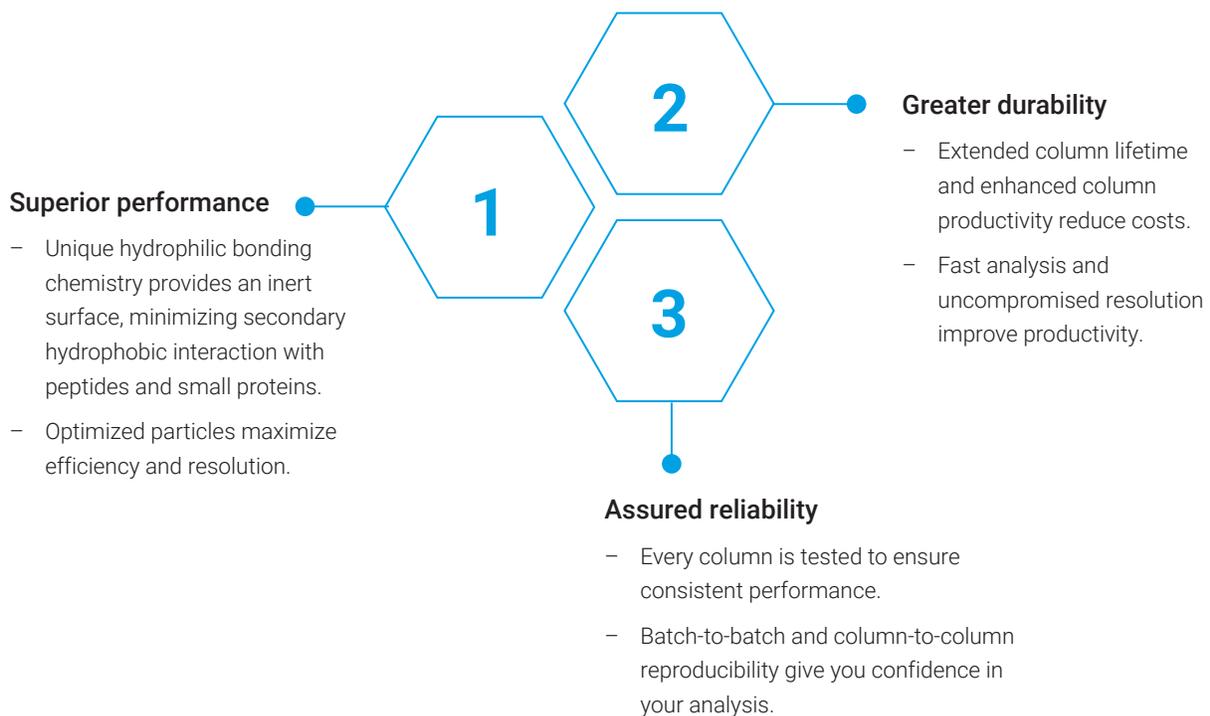


# Optimize Your SEC Analysis with High Resolution and Reduced Secondary Interactions

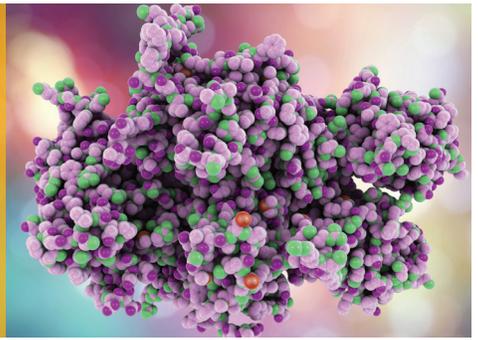
Size exclusion chromatography (SEC) is a reference method for the analysis of protein aggregates. These aggregates are critical quality attributes (CQAs) because they may alter the potency, safety, or immunogenicity of biotherapeutic proteins and peptides.

Agilent AdvanceBio SEC 120 Å 1.9 µm columns are the newest members of the AdvanceBio SEC product line, and are suitable for UHPLC and HPLC instruments. They deliver resolution, inertness, and faster separation for small therapeutic proteins and peptides, so you can identify product-related size variants qualitatively and quantitatively.

## Optimized sub-2 µm particles give you these advantages:



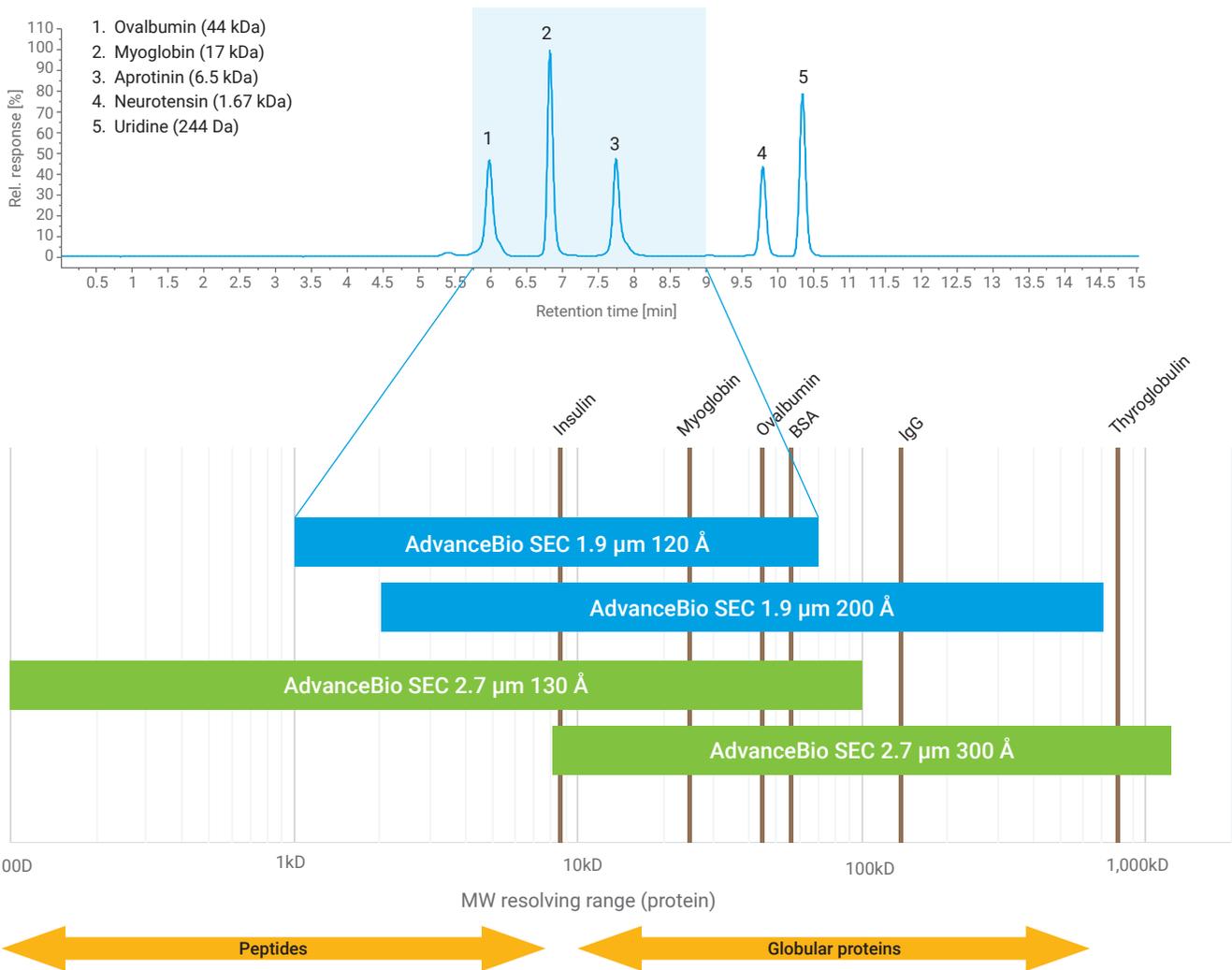
# Superior Results for Peptides and Small Proteins



## High resolution, high-efficiency separations, and an inert surface

The 1.9  $\mu\text{m}$  silica particles feature optimized pore size and volume to ensure high resolution and separation efficiency. In addition, the column surface is modified by proprietary hydrophilic bonding chemistry to minimize nonspecific interactions with peptides and small therapeutic proteins.

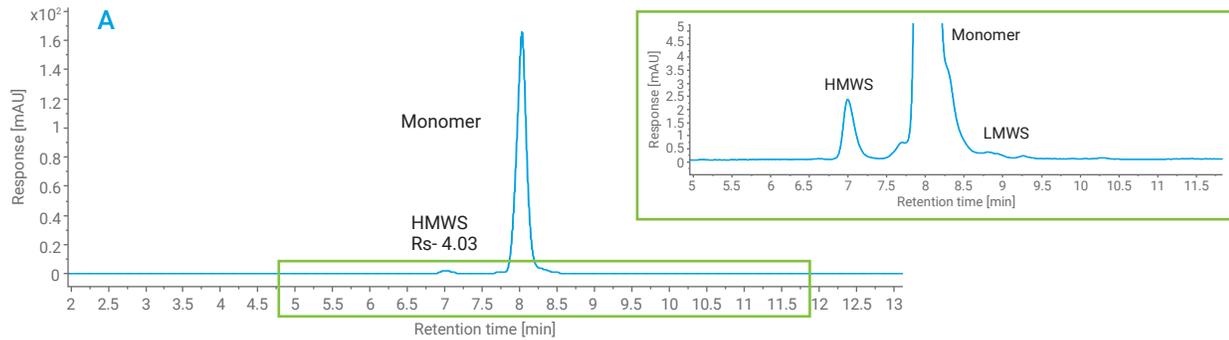
## Superior resolution and efficient separation for aggregate and fragment analysis



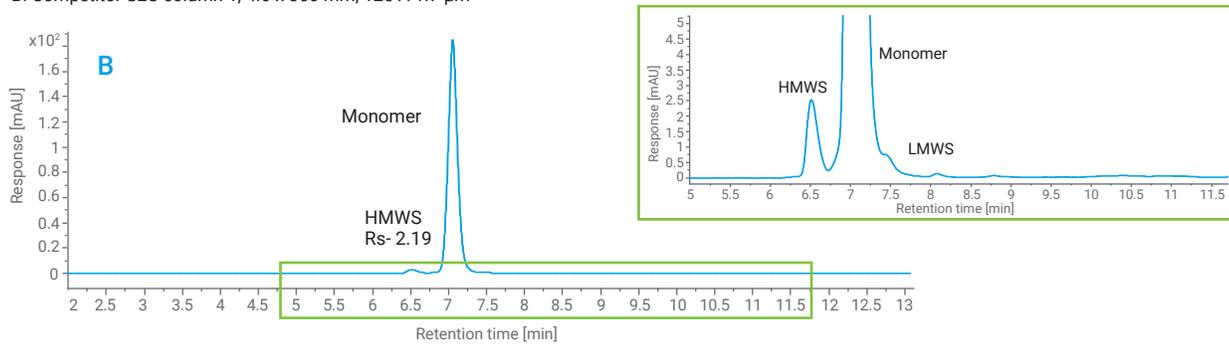
AdvanceBio SEC 120  $\text{\AA}$  1.9  $\mu\text{m}$  provides highly optimized performance for peptides and small proteins.

**AdvanceBio SEC 120 Å 1.9 µm columns demonstrate better resolution and separation efficiency, compared to competitor columns.**

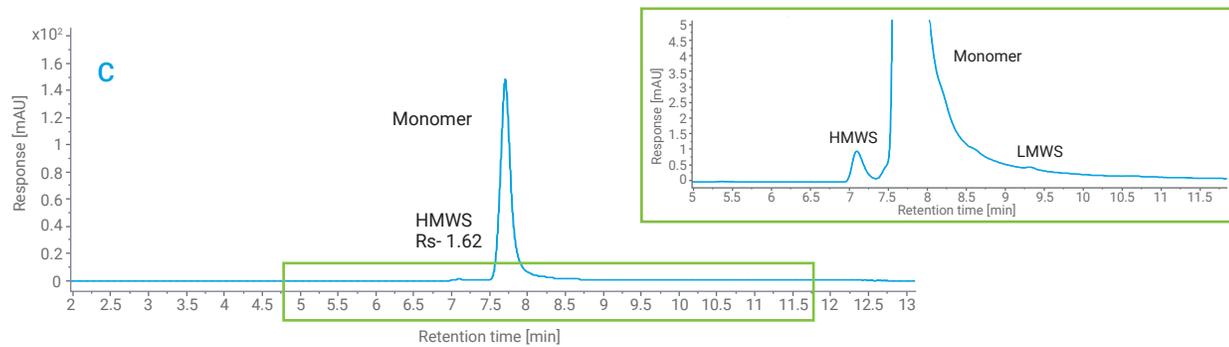
A: Agilent AdvanceBio SEC, 4.6 x 300 mm, 120 Å 1.9 µm



B: Competitor SEC column 1, 4.6 x 300 mm, 125 Å 1.7 µm



C: Competitor SEC column 2, 4.6 x 300 mm, 150 Å 1.8 µm



<b>Instrument:</b>	Agilent 1260 Infinity II Bio-inert LC system	<b>Sample:</b>	Heat-stressed human insulin (60 °C for 6 hours); concentration 4 mg/mL
<b>Software:</b>	Agilent OpenLab CDS	<b>Temperature:</b>	25 °C
<b>Flow rate:</b>	0.30 mL/min	<b>Injection volume:</b>	2 µL
<b>Eluent:</b>	Arginine (1.0 g/L)/acetic acid/acetonitrile (65/15/20, v/v/v)	<b>Detection:</b>	VWD, 276 nm

Size-exclusion chromatograms of stressed insulin with aggregates and low molecular weight fragments

A: Agilent AdvanceBio SEC, 120 Å 1.9 µm

	Area	%Area	Peak Tailing	Peak Width
HMWS	31.3	1.93		
Insulin monomer	1587.5	97.66	1.0	0.13
LMWS	6.7	0.41		

B: Competitor SEC column 1, 125 Å 1.7 µm

	Area	%Area	Peak Tailing	Peak Width
HMWS	25.7	1.57		
Insulin monomer	1601.7	97.85	1.10	0.13
LMWS	9.5	0.58		

C: Competitor SEC column 2, 150 Å 1.8 µm

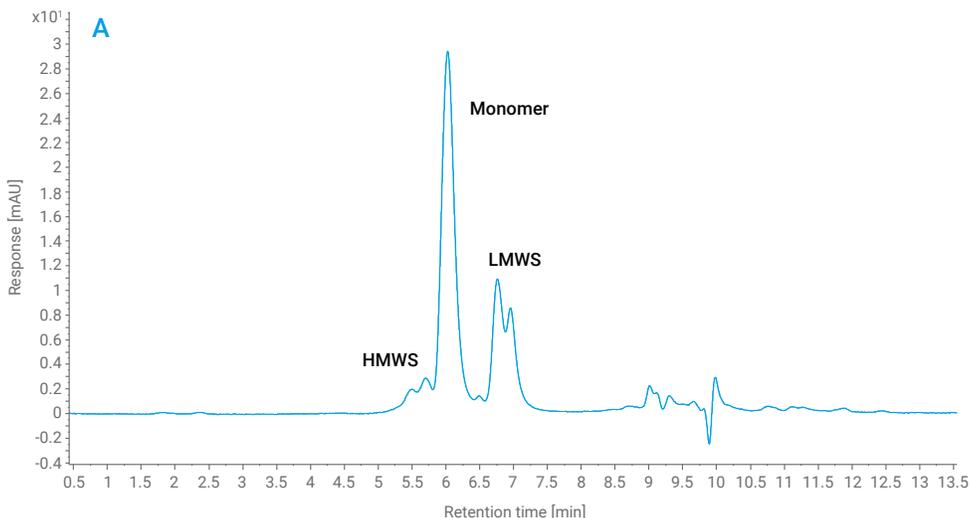
	Area	%Area	Peak Tailing	Peak Width
HMWS	16.5	1.10		
Insulin monomer	1624.0	98.80	1.37	0.14
LMWS	3.3	0.20		

## Inert stationary surface ensures excellent peak shape and minimizes nonspecific interactions

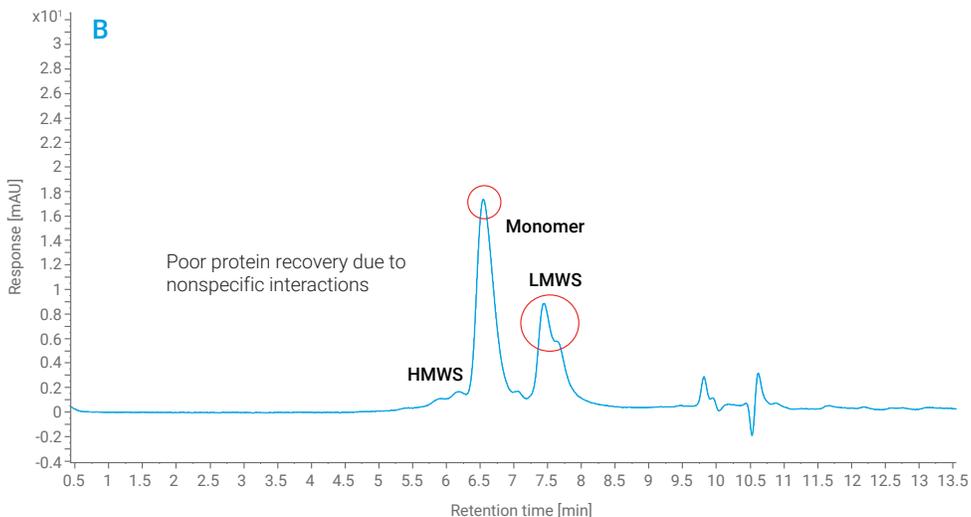
Secondary interactions make it difficult to characterize samples. They prolong sample elution time and cause poor peak shape and separation from differences. AdvanceBio SEC 120 Å 1.9 µm columns prevent secondary interactions with “sticky” proteins and peptides, demonstrating better peak shape and area recovery than the competition.

### Size exclusion chromatogram of human growth hormone

A: Agilent AdvanceBio SEC, 4.6 x 300 mm, 120 Å 1.9 µm



B: Competitor SEC column, 4.6 x 300 mm, 125 Å 1.7 µm



**Instrument:** Agilent 1260 Infinity II Bio-inert LC system  
**Software:** Agilent OpenLab CDS  
**Flow rate:** 0.35 mL/min  
**Eluent:** 150 mM phosphate, pH 7  
**Sample:** Concentration 1 mg/mL  
**Temperature:** 25 °C  
**Injection volume:** 2 µL  
**Detection:** UV, 220 nm

Column	Monomer RT (min)	Total Peak Area (Avg n=2)	Monomer Peak Tailing	Monomer Peak Width
Agilent AdvanceBio SEC	6.02	691.81	1.22	0.21
Competitor SEC column 1.7 µm	6.54	581.10	1.33	0.28

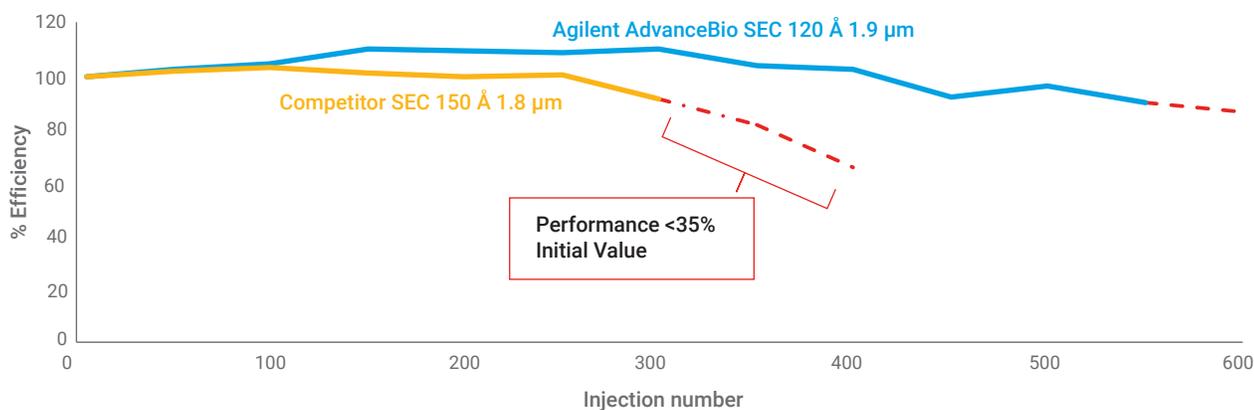
# Improved Column Lifetime Reduces Cost



## Extended lifetime: a smart investment in your lab

We engineered our 1.9  $\mu\text{m}$  monodisperse silica particles for best-in-class mechanical strength and improved column lifetime. During development, columns were tested using a flow stop/start procedure, which is designed to mimic typical use in demanding laboratory environments.

AdvanceBio SEC 120  $\text{\AA}$  1.9  $\mu\text{m}$  columns showed less than 10% drop in uridine plate number over 550 injections, confirming excellent mechanical stability.

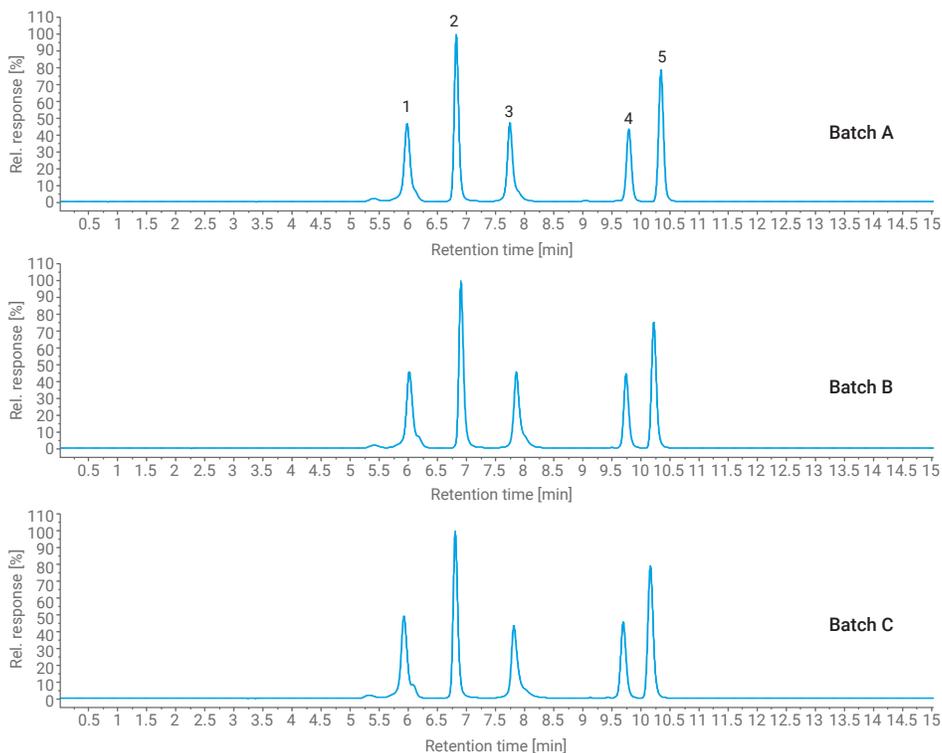


**Instrument:** Agilent 1260 Infinity II Bio-inert LC system  
**Column:** 4.6 x 300 mm  
**Eluent:** 100 mM phosphate, pH 6.8  
**Flow rate:** 0.30 mL/min  
**Temperature:** 25  $^{\circ}\text{C}$   
**Detection:** UV, 280 nm  
**Sample:** BioRad protein mix and uridine (stop flow every 50 injections)

## Proven reliability to meet your quality control needs

Developed and manufactured by Agilent, AdvanceBio SEC 120 Å 1.9 µm columns are tested during each process step to ensure quality, robustness, and performance. In addition, Agilent AdvanceBio SEC method validation kits are available to simplify validation and help you meet regulatory requirements.

Batch-to-batch quality control ensures reproducibility and robustness.



**Instrument:** Agilent 1260 Infinity II Bio-inert LC system  
**Software:** Agilent OpenLab CDS  
**Column:** 4.6 x 300 mm  
**Eluent:** 150 mM phosphate, pH 7  
**Flow rate:** 0.35 mL/min  
**Temperature:** 25 °C  
**Detection:** UV, 220 nm

	Protein Mix Standard	Molecular Weight (Da)
1	Ovalbumin	44,000
2	Myoglobin	17,000
3	Aprotinin	6,700
4	Neurotensin	1,700
5	Uridine	244

## Ordering information

Description	Part Number
AdvanceBio SEC 120 Å, 1.9 µm, 4.6 x 300 mm	PL1580-5250
AdvanceBio SEC 120 Å, 1.9 µm, 4.6 x 150 mm	PL1580-3250
AdvanceBio SEC 120 Å, 1.9 µm guard, 4.6 x 30 mm	PL1580-1250
AdvanceBio SEC 120 Å, 1.9 µm, 4.6 x 300 mm, Method Validation Kit	PL1580-5250K
AdvanceBio SEC 120 Å, 1.9 µm, 4.6 x 150 mm, Method Validation Kit	PL1580-3250K

Download the AdvanceBio SEC 1.9 µm column Quick Start Guide at [www.agilent.com/chem/advancebiosec](http://www.agilent.com/chem/advancebiosec)

## Agilent CrossLab services

CrossLab is an Agilent capability that integrates services and consumables to support workflow success and important outcomes like improved productivity and operational efficiency. Through CrossLab, Agilent strives to provide insight in every interaction to help you achieve your goals. CrossLab services include method optimization, flexible service plans, and training for all skill levels. We have many other products and services to help you manage your instruments and your lab for best performance.

Learn more about Agilent CrossLab, and see examples of insight that leads to great outcomes, at [www.agilent.com/crosslab](http://www.agilent.com/crosslab)

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